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Alzheimer Disease and Associated Disorders
Vol. 14, Suppl. 1, pp. S39–S46

Neurotrophic Factor Strategies for the Treatment of Alzheimer Disease

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Summary: Cholinergic neurons in the nucleus basalis of Meynert are reduced early in the course of Alzheimer disease, and the dysfunction of cholinergic neurons is believed to be primarily responsible for cognitive deficits in the disease. Nerve growth factor has a trophic effect on cholinergic neurons and therefore may have some beneficial effects on the cognitive impairment observed in patients with Alzheimer disease. Experimental studies demonstrated that a continuous infusion of nerve growth factor into the cerebroventricle prevents cholinergic neuron atrophy after axotomy or associated with normal aging and ameliorates cognition impairment in these animals. A clinical study in three patients with Alzheimer disease revealed, however, that a long-term intracerebroventricular infusion of nerve growth factor may have certain potentially beneficial effects, but the continuous intracerebroventricular route of administration is also associated with negative side effects that appear to outweigh the positive effects. Several other strategies have been suggested to provide neurotrophic support to cholinergic neurons. In this article, we review the neurotrophic factor strategies for the treatment of Alzheimer disease. **Key Words:** Alzheimer disease—Nerve growth factor—Brain-derived neurotrophic factor—Neurotrophin.

Alzheimer disease (AD) is a neurodegenerative disorder that is neuropathologically characterized by the presence of numerous senile plaques and neurofibrillary tangles accompanied by neuronal loss. The extracellular senile plaques are composed of amyloid β-peptides (Aβ), 40–42 amino acid peptide fragments of the β-amyloid precursor protein (APP), whereas the intracellular neurofibrillary tangles are composed of highly phosphorylated tau proteins (Selkoe, 1994; Yankner, 1996; Hardy, 1997). Cholinergic neurons in the nucleus basalis of Meynert are reduced early in the course of AD, and the dysfunction of cholinergic neurons is believed to be primarily responsible for cognitive deficits in the disease (Coyle et al., 1983). Clinical manifestations of AD are primarily the progressive loss of memory and language. With disease progression, patients may have psychiatric and behavioral disturbances (Katzman, 1986).

It has been proposed that neuronal atrophy and death in neurodegenerative disorders including AD are the result of a lack of trophic support (Appel, 1981). It becomes evident that there are many growth factors in the brain that fulfill a functional definition of neurotrophic factors (NTF). NTF are the proteins that regulate survival and differentiation of neuronal cells (Hefti, 1997). Members of the nerve growth factor (NGF) family (neurotrophins) have been cloned and characterized in mammals; NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5 (Barde, 1996). There are two classes of cell surface receptors for neurotrophins: low-affinity neurotrophin receptors (p75NTR, 75-kDa proteins interacting with all neurotrophins) and high-affinity tyrosine kinase receptors of the trk family (trkA, B, and C). NGF interacts with trkA, whereas trkB binds both BDNF and NT-4/5. NT-3 acts as a specific ligand for trkC. Consistent with the restricted neuronal

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specificity of NGF, trkA is localized to only a very few neuronal types in the central nervous system (CNS) and peripheral nervous system (PNS). On the other hand, trkB and trkC are widely distributed throughout the brain (Chao, 1992). Therefore, the actions of BDNF, NT-3, and NT-4/5 in the CNS are much more diverse than those of NGF. Several lines of evidence suggest that NGF and other members of the neurotrophin family have potential as therapeutic agents for the treatment of AD (Hefti, 1997; Dragunow et al., 1998).

RATIONALE FOR NTF STRATEGIES IN AD

Several experimental studies support the use of NGF for the treatment of AD: NGF is a potent and selective neurotrophic factor for the basal forebrain cholinergic neurons (Honegger and Lenoir, 1982; Gnahn et al., 1983; Seiler and Schwab, 1984; Korsching et al., 1985; Sheldon and Reichardt, 1986). The high-affinity NGF receptor trkA is abundantly expressed in the basal forebrain cholinergic neurons (Vazquetz and Ebendahl, 1991; Holtzman et al., 1992). NGF prevents cholinergic neuron atrophy and cognition impairment after axotomy or associated with normal aging in animals (Hefti, 1986; Williams et al., 1986; Fischer et al., 1987; Kromer, 1987; Tuszynski et al., 1991). We demonstrated that a continuous infusion of a specific Fab′ fragment of anti-NGF antibody into the cerebral ventricle causes learning and memory impairment in a water maze task and alters nuclear morphology in the hippocampus and cortex (Nabeshima et al., 1991). Furthermore, Nitta et al. (1993) found a significant impairment of passive avoidance learning and a marked reduction of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities in the hippocampus of rats that had previously been infused with anti-NGF antibody into the septum (Nitta et al., 1993*c*). Our group also showed that a continuous infusion of NGF ameliorated learning and memory impairment in rats with basal forebrain lesions (Ogawa et al., 1993). These results suggest that NGF regulates cholinergic functions, which play an important role in learning and memory. On the other hand, NGF has been shown to upregulate expression of APP, which in turn could accelerate deposition of Aβ in the brain (Mobley et al., 1988; Butcher and Woolf, 1989). It was recently demonstrated, however, that targeted intraparenchymal delivery of human NGF by gene transfer to the basal forebrain for 3 months does not accelerate Aβ plaque deposition in rhesus monkeys (Tuszynski et al., 1998). Therefore, it is less likely that NGF treatment accelerates the progress of AD.

BDNF also improves the survival and differentiated functions of septal cholinergic neurons in vitro (Anderson et al., 1990) and prevents the degeneration of medial cholinergic neurons after fimbria transection (Morse et al., 1993). In addition to such long-term neurotrophic effects, BDNF rapidly enhances synaptic transmission and modulates neurotransmitter release (Levine et al., 1995). Furthermore, it has been demonstrated that learning and memory and hippocampal long-term potentiation (LTP) are impaired in BDNF mutant mice (Korte et al., 1995; Linnarsson et al., 1997). An increase in BDNF mRNA expression in the hippocampus was associated with the water maze learning (Kesslak et al., 1998). Recently, we observed that a continuous intracerebroventricular (ICV) infusion of antisense BDNF oligodeoxynucleotides results in an impairment of reference and working memory in a radial arm maze (Yamada et al., unpublished observation). Therefore, in addition to having neuroprotective effects, BDNF is involved in synaptic plasticity and memory processes, the effects being particularly relevant to the treatment of AD.

ALTERATIONS OF NEUROTROPHINS AND THEIR RECEPTORS IN AD

The levels of NGF and other neurotrophins, as well as their receptor contents, in the brains of AD patients have been measured to see whether a lack of neurotrophic factors could contribute to the development of AD. The results of these studies indicated that NGF levels in the postmortem AD brains are unchanged (Goedert et al., 1986; Allen et al., 1991; Murase et al., 1993; Jette et al., 1994) or even increased (Crutcher et al., 1993; Scott et al., 1995; Narisawa-Saito et al., 1996) compared with those found in the neurologically normal control. In contrast, expression of the high-affinity NGF receptor trkA protein in the nucleus basalis of Meynert (NBM) was markedly reduced (Salehi et al., 1996; Boissiere et al., 1997b; Mufson et al., 1997). Regarding alterations in the expression of low-affinity NGF receptors, the results are controversial (Goedert et al., 1989; Higgins and Mufson, 1989; Treanor et al., 1991; Strada et al., 1992) A loss of high-affinity NGF receptors trkA in the cholinergic neurons may result in a decrease in reduced retrograde transport of NGF from the cortex to the NBM, which may cause the selective neurodegeneration observed in AD.

BDNF protein and mRNA levels were reported to be decreased in the brains of AD patients (Phillips et al., 1991; Murray et al., 1994; Connor et al., 1997), although changes in the high-affinity BDNF receptor trkB are inconsistent (Connor et al., 1996; Salehi et al., 1996, 1998; Boissiere et al., 1997*a*). Therefore, deficits of neurotrophic effect of BDNF may also be involved in the

development of AD. Regarding alteration of NT-3 expression in AD, two studies reported no changes (Phillips et al., 1991; Murase et al., 1994), whereas a significant reduction of NT-3 protein levels in the motor cortex was reported by Narisawa-Saito et al. (1996).

CLINICAL EFFECTS OF NGF IN AD

Clinically, three AD patients were treated with β-NGF (purified from male mouse submandibular glands), administered continuously into the lateral cerebral ventricle for 3 months (total dose, 6.6 mg) in the first two patients and for three shorter periods (total dose, 0.55 mg) in the third patient (Jönhagen et al., 1998). The results in the first patient showed a marked transient increase in nicotine binding and a persistent increase in cerebral blood flow, as well as a progressive decrease of slow wave EEG activity. After 1 month of NGF infusion, tests of verbal episodic memory were improved, whereas other cognitive tests were not (Olson et al., 1992; Seiger et al., 1993). From these results, it was suggested that NGF counteracts cholinergic deficits in AD. Unfortunately, in subsequent clinical research no clear cognitive amelioration was demonstrated in any patients. An increase in nicotine binding was found in some brain areas in these patients 3 months after the end of NGF treatment. The amount of slow-wave cortical activity was reduced in the first two patients, but not the third. The cerebral blood flow was improved in the first patient, but not the other two patients. Two negative side effects, a dull constant back pain and a marked weight loss, accompanied NGF treatment. It was concluded that long-term ICV NGF administration may cause certain potentially beneficial effects, but the ICV route of administration is also associated with negative side effects that appear to outweigh the positive effects (Jönhagen et al., 1998).

NTF STRATEGIES FOR THE TREATMENT OF AD

The neurotrophic hypothesis for neurodegenerative disease has prompted basic and clinical studies into the clinical use of NTF as a therapeutic agent for the treatment of neurodegenerative disease including AD. Since NTF proteins cannot be delivered orally and do not cross the blood-brain barrier (BBB), several means of delivering NTF proteins into the brain, as well as other alternative ways to provide neurotrophic support, have been proposed (Table 1).

Direct delivery of NTF proteins into the brain would bypass the BBB and increase NTF levels in the brain. Cannulation into the cerebroventricular space and long-term infusion of NGF protein via the cannula were used

TABLE 1. *Neurotrophic factor (NTF) strategies for the treatment of Alzheimer disease*

1. Direct delivery of NTF to the brain
   a. Long-term intracerebroventricular infusion of NTF after cannulation
   b. Slow-releasing implants of NTF embedded in a biodegradable polymer matrix
2. Carrier-mediated NTF delivery across the blood-brain barrier into the brain
3. NTF gene therapy
4. Regulation of NTF synthesis
5. NTF receptor agonists
6. Regulation of NTF signaling
7. Neuroimmunophilin ligands

in the clinical trials for AD. It became apparent that a continuous ICV NGF infusion is not tolerated well by patients because of severe back pain as described (Jönhagen et al., 1998). The side effects may result from activation of NGF receptors that are present on Schwann cells and sympathetic and sensory axons (Blesch et al., 1998). Accordingly, NGF should be administered to the basal forebrain region in a highly regionally restricted manner. Slow-releasing implants that contain the active protein embedded in a biodegradable polymer matrix (NGF-containing polymers) could be implanted into the selected brain area in one operation (Hoffman et al., 1990). Therefore, this method may reduce the risk of infection and side effects of ICV infusion of NGF.

Brain delivery of exogenous NTF may also be achieved by conjugating it to antibodies to transferrin receptors or by gene therapy. It has been demonstrated that the conjugated NGF crosses the BBB and exerts the trophic effects in vivo (Friden et al., 1993). Gene therapy can deliver a single molecule or several well-defined molecules in a highly specific spatial and temporal fashion (Blesch et al., 1998). It includes ex vivo and in vivo approaches. In ex vivo gene transfer, cells genetically modified to secrete a certain protein are grafted into a discrete area of the brain. The in vivo approach is a direct gene transfer into the brain by the delivery of transgenes to the host cells. Many studies focused on the ex vivo approach because of its ability to generate various types of modified cells that can be characterized in vitro before use. For instance, transplantation of fibroblasts, modified to secrete NGF, into the basal forebrain was shown to protect cholinergic neurons and promote functional recovery after fimbria-fornix transection (Rosenberg, 1988). Cholinergic neuron atrophy with aging can also be ameliorated by grafting NGF-producing cells (Chen and Gage, 1995). As an example for the in vivo gene therapy, intrastriatal injection of an adenoviral vector expressing glial cell-line–derived neurotrophic factor

(GDNF) is reported to prevent dopaminergic neuron degeneration and behavioral impairment in a rat model of Parkinson's disease (Bilang-Bleuel et al., 1997).

REGULATION OF ENDOGENOUS NTF SYNTHESIS

NTF expression can be regulated by both developmental and environmental stimuli. A variety of endogenous and exogenous compounds was demonstrated to induce NGF expression in cultured cells and brain and peripheral tissues. For example, interleukin-1β (Lapchack, 1993) and testosterone (Katoh-Semba et al., 1990) are involved in the regulation of NTF expression. Low molecular weight NGF inducers were reported as candidates of antidementia drugs. Those include catechol derivatives such as 4-methylcatechol (Furukawa et al., 1989; Carswell et al., 1992), benzoquinones (Takeuchi et al., 1990; Nitta et al., 1993*b*), purine derivatives including propentofylline and AIT-082 (Shinoda et al., 1990; Glasky et al., 1997), hericenones from the mushroom *Hericium erinaceum* (Kawagishi et al., 1991), sesquiterpene-neolignans from *Magnolia obovata* (Fukuyama et al., 1992), pyrroloquinolines (Yamaguchi et al., 1993), erinacines from the mycelia of *Hericium erinaceum* (Kawagishi et al., 1994) and diterpenoids isolated from *Euphorbia* such as kansuinin A, ingenol triacetate, and jolkinolide B (Yamaguchi et al., 1994).

We propose that orally active NGF synthesis stimulators have potential as therapeutic agents in AD and may have some advantages compared with NGF itself in terms of quality of life for the patient, since such treatment does not require the insertion of an NGF delivery catheter into the brain (Yamada et al., 1997; Nabeshima and Yamada, in press). We investigated the effects of idebenone and propentofylline as prototypes of NGF synthesis stimulators. These two compounds, partially restored the age-associated decrease of NGF levels in the frontal and parietal cortices (Nabeshima et al., 1993, 1994; Nitta et al., 1993*a*), and ameliorated learning and memory impairments induced by bilateral forebrain lesions (Fuji et al., 1993; Nitta et al., 1994), a continuous infusion of anti-NGF antibody into the septum (Nitta et al., 1996) and a continuous ICV infusion of Aβ (Yamada et al., 1998, 1999*b*).

NTF RECEPTOR AGONISTS

NTF could be replaced by active peptide fragments or molecules that mimic the active sites of NTF. Although there are two classes of NGF receptors, low-affinity p75NTR and high-affinity trkA, it has been demonstrated that activation of trkA is sufficient to rescue axotomized cholinergic neurons in vivo by using polyclonal antibodies that act as specific agonists of trkA (Lucidi-Phillipi et al., 1996). Small monomeric cyclic analogs of NGF were found to be competitive trkA antagonists (LeSauteur et al., 1995). Furthermore, the NGF mimetic trkA antagonist C(92-96) caused a significant decrease in the number and size of vesicular acetylcholine transporter sites, supporting the notion that endogenously produced NGF acting through trkA receptors is involved in the maintenance of the cholinergic phenotype in a normal adult rat brain (Debeir et al., 1999). As an example of small nonpeptidyl NTF agonists, SB247464 was demonstrated to act as a granulocyte colony–stimulating factor (G-CCF) agonist and elevate peripheral blood neutrophil counts in mice (Tian et al., 1998).

REGULATION OF NTF SIGNALING

Ligand-induced oligomerization of receptor protein tyrosine kinases and autophosphorylation has been established as a general mechanism for the activation of growth factor receptors. Trks activated by ligand binding stimulate intracellular signaling, activating a variety of enzymes and effectors including phospholipase C-γ (PLCγ), phosphatidylinositol 3-kinase (PI3-K), Shc. Grb2, small GTP binding proteins (Ras. Rac, and cdc42). and mitogen-activated protein kinases (MAPK) (Segal and Greenberg, 1996; Skaper and Walsh, 1998). Recent studies with PC12 cells demonstrated that NGF exerts a survival-promoting effect via activation of the PI3-K pathway and promotes neuronal differentiation through the Ras-MAPK pathway (Marshall, 1995; Yao and Cooper, 1995; Franke et al., 1997; Dudek et al., 1997). Although the exact mechanisms are unclear, some compounds such as SR57746A (Pradines et al., 1995) and Triap (Paul and DaVanzo, 1992) have been shown to potentiate the effects of NGF in PC12 cells. It is possible that these compounds activate the NTF signaling pathway without affecting NGF receptors.

Neuronal survival is also negatively regulated by the activation of a signaling cascade responsive to stress and injury. In neuronally differentiated PC12 cells, withdrawal of NGF causes apoptosis that is preceded by a decrease of extracellular signal-regulated kinase (ERK) activity and an increase in stress-activated kinases such as c-Jun N-terminal kinase (JNK) and p38 (Xia et al., 1995). The JNK and p38 kinases are activated by proinflammatory cytokines, hyperosmolarity, heat shock, endotoxin, and other cellular stresses. The activation cascades for JNK and p38 appear to be distinct. The MAPK kinase MEK4 activates JNK but not p38, whereas MEK3 and MEK6 activate p38 but not JNK (Skaper and Walsh, 1998). It has been shown that a specific inhibitor of the

JNK signaling pathway, CEP-1347 (KT7515), can promote long-term survival of cultured chick embryonic dorsal root ganglion, sympathetic, ciliary, and motor neurons (Borasio et al., 1998). CEP-1347 is also reported to rescue motoneurons undergoing apoptosis in the absence of trophic support (Maroney et al., 1998). Regarding the role of p38, it has been demonstrated that a specific p38 inhibitor PD169316 blocked the increase in p38 activity and apoptosis induced by serum deprivation in Rat-1 fibroblasts and by NGF withdrawal in differentiated PC12 cells (Kummer et al., 1997). Other p38 inhibitors, the pyridinyl imidazole compounds SB203580 and SB202190, also promoted the in vitro survival of sensory, sympathetic, ciliary, and motor neurons (Horstmann et al., 1998). These results suggest that intervention in the JNK and/or p38 signaling cascade offers opportunities for the development of therapeutic agents for neurodegenerative diseases.

NEUROIMMUNOPHILIN LIGANDS

Immunophilins are the receptor proteins for the major immunosuppressant drugs cyclosporin A, FK506, and rapamycin. Immunophilins are more abundant in the nervous system than in the immune systems and may modulate neuronal function (Snyder et al., 1998). There is evidence that immunophilin ligands are neurotrophic for numerous classes of damaged neurons both in culture systems and intact animals. Furthermore, the immunosuppressive properties of FK506 and cyclosporin A can be functionally dissociated from their neurotrophic effects (Steiner et al., 1997*a*). One of these compounds, GPI-1046, has neuroprotective effects in a number of CNS degenerative models including the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine models of Parkinson's disease (Steiner et al., 1997*b*). Further studies are needed to examine the effects of immunophilin ligands on neurodegenerative disease including AD.

CONCLUSIONS

In this review, we discussed possible neurotrophic factor strategies for the treatment of AD. Evidence suggests that loss of trophic support (deficiency of trkA and/or BDNF) may occur in AD, although it is difficult to determine whether the loss of NTF is the cause or the result of the neuropathology. A clinical study with three AD patients who received ICV infusion of NGF demonstrated that side effects such as back pain appear to outweigh the positive effects. Therefore, a means of delivering NTF into the brain other than direct ICV infusion is needed to assess the clinical effects of NTF therapy in AD. The second-generation strategies of NTF therapy in AD include slow-releasing implants that contain active NTF proteins embedded in a biodegradable polymer matrix, carrier-mediated delivery of NTF across the BBB, and NTF gene therapy. Alternatively, small molecules that pass the BBB and regulate NTF synthesis, activate NTF receptors, or modulate the NTF signaling for survival and differentiation would be suitable for a noninvasive NTF pharmacotherapy in AD. We believe that the combination of NTF therapy with other pharmacotherapies including cholinergic, anti-inflammatory, antioxidant, and antiamyloid therapies (Nabeshima and Yamada, in press; Yamada et al., 1999*a*) would provide the best hope for the treatment of AD.

Acknowledgments. The authors thank Drs. A. Nitta, A. Itoh, Y. Ogihara, J. Onishi, M. Mizuno, T. Fukuta, T. Hasegawa, Y-P. Tang, Y. Murata, H. Seo, Y. Robitaille, R. Ouirion, K. Fuji, S. Ogawa, T. Kameyama, K. Murase, R. Takeuchi, Y. Furukawa, K. Hayashi, and M. Ogawa for their contributions to this research project. This study was supported in part by grants-in-aid for Science Research from the Ministry of Education, Science, Sports and Culture of Japan (nos. 07557009, 10897005, and 97450), a Smoking Research Foundation grant for biomedical research, grants from the Suzuken Memorial Foundation, and the Research Foundation for Pharmaceutical Sciences, the Center of Excellence Grant.

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Alzheimer Disease & Associated Disorders

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LIPPINCOTT WILLIAMS & WILKINS

Volume 14, Supplement 1, 2000